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Influence of testis state, temperature and delay in semen collection on spermatozoa motility in the cultured Siberian sturgeon (*Acipenser baeri* Brandt)

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Abstract

A factorial design was set up to test the effects of three factors on spermatozoa motility in cultured Siberian sturgeons, *Acipenser baeri*, removed from 10°C water temperature. From the four tested temperatures (10°C, 12.5°C, 15°C and 17.5°C) during hormonal treatment, the significantly highest spermatozoa motilities (65%) were obtained at 10°C and the lowest (41%) at 17.5°C. The increasing delay in semen collection gave the following results: 24 h (30% motility), 36 h (72%), 48 h (64%) and 60 h (53%). Males of which the testis state was referred to as firm or soft gave significantly better spermatozoa motilities (70%) as compared with viscous or liquid testis (40%). Semen pH, osmotic pressure and spermatocrit did not show any relationship with spermatozoa motility.

Keywords: Spermatozoa; Sturgeon; Semen; Temperature; Testis

1. Introduction

The reproduction of cultured sturgeons is based on hormonal stimulation to induce ovulation and spermiation. The results depend on the accuracy in final selection of those

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breeders able to give the best response in terms of quantity and quality, the applied treatment, and the management of breeders from rearing facilities into hatchery reproductive tanks. Fewer studies have been made on males than on females. Preliminary assessments in grading males by testis appearance have been reported (Conte et al., 1988; Williot, unpublished data), but there have been no studies relating the testis developmental state and semen quality. The current practice consists in stimulating the males just after the females (Conte et al., 1988; Williot, unpublished data), but no study has examined the effect of semen collection delay after hormonal treatment. With regard to water temperature, Sokolov and Malyutin (1977) suggested an optimal spawning range of 11–16°C in the Lena River (Russia) where spawning occurred in late spring–summer time, and artificial propagation has been reported within the range 11–20°C (Khakimoullin, 1979; Williot et al., 1991), but there is no experimental work dealing with this factor.

As the interval between two successive ovarian cycles shows different patterns in cultured Siberian sturgeons, the selection of breeders requires special attention. In practice, this is organised in two steps, the first being a pre-selection carried out in November. Ovarian follicles are sampled via incision. A minimum diameter of 2.8 mm allows separation of the spawnable females in the following few months (Williot and Brun, 1998). A piece of testis tissue is surgically sampled and observed for its hardness. In contrast to the females, the reproductive potential of which will again be determined just before the spawning operation, the males are not subjected to a second selection. This means that males which showed a softer testis in November are directly injected without knowing the consequences on semen quality.

As a result of this lack of a reliable procedure in males, more males than necessary are injected as a precaution, and a pool of the best semen, judged on spermatozoa motility, is used to fertilize the eggs (Ginsburg and Dettlaff, 1969; Conte et al., 1988; Williot et al., 1991).

Due to the late puberty, the maintenance of male broodstock is costly, so new approaches are needed to optimise their management.

The objectives of this work were: (1) to study simultaneously the influence of the following three factors on semen quality, (judged by spermatozoa motility): maturity state of testis, water temperature during hormonal treatment, and waiting period for semen collection; and (2) to search for a relationship between spermatozoa motility and some semen characteristics.

2. Materials and methods

2.1. Fish farming

The experimental fish belonged to the 1984 hatchery cohort from broodstock originating from wild Siberian sturgeons from Lena River. The sex was determined in 1993 by observing a piece of gonad obtained via a small abdominal incision closed with cross-stitches. Females were detected by presence of oocytes under stereomicroscope

(×16) and males by spermatozoa under the microscope (×400) and the fish were appropriately tagged. They were reared in raceways, supplied by river water with oxygen content maintained at a minimum level of 90% saturation. The fish were fed a commercial extruded trout diet at a daily rate of 0.1–0.7% of body weight according to water temperature.

2.2. Experimental design

In late October 1994, tagged males were weighed and subjected to a small abdominal cut for testis sampling. The gonad state was observed for its consistency by eye and by feel. It was classified into four categories: liquid (L), viscous (V), soft (S) and firm (F). We chose four temperature levels, 10°C, 12.5°C, 15°C and 17.5°C, using one temperature in each of four tanks ($\phi = 2$ m). The lowest temperature was the river water at the time of the experiment and the highest, that of underground water previously completely gas–degassed and then oxygen-enriched up to 90% saturation. The two intermediate temperatures were obtained by mixing these two sources in appropriate proportions. The water renewal was regulated to maintain an outflow oxygen content of 90% saturation. Ovulation latency decreases from about 55 h at 10°C (Williot, unpublished data) to about 22 h at 20°C according to Khakimoullin (1979), so we sampled at 24, 36, 48 and 60 h after injection.

Each of the four tanks contained eight fish (a total of 32 fish), two of each testis state randomly chosen. At a given collection time, we sampled the four types of testis state males, two in each tank. Each of these males was sampled once. As a result, we had a complete equilibrated Latin Square design with three factors, each one at four levels.

2.3. Induced spermiation, semen collection and characteristics

The fish were brought from outside raceways (water temperature 10°C) to the hatchery. Immediately upon arrival, they were stimulated with commercial acetone-dried carp pituitary homogenate (Argent City, USA) at 2 mg/kg body weight (Williot et al., 1991). They were identified by a colour mark for each gonad state attached to their tag to avoid extra handling, and the fish were then distributed in each of the four tanks.

At sampling time, the male fish were held on their back in a V-shaped operating table, wiped around the urogenital area, and a dry flexible polypropylene tube (5 and 3 mm external and internal diameter, respectively) was carefully introduced into the genital opening allowing complete semen collection in beakers when accompanied by a gentle abdominal massage. Volume of semen was estimated to the nearest 5 ml, pH (Schott gerate) to the nearest 1/100. Osmotic pressure was measured in duplicate by cryoscopy (Knauer osmometer) to the nearest 5 mosM/kg. Spermatocrit was measured in triplicate after semen sub-samples centrifugation (10 mn at 3000 rpm) in standard hematocrit tubes. Motility was estimated under a microscope (×400) as a percentage 30 s after dilution of semen in water in the ratio 1:40. This visual estimation takes into account an estimation of the number of motile spermatozoa, their speed and type of

Table 1
The protocol and spermatozoa motility of 16 cultured Siberian sturgeons that were analysed in this study

Weight (kg)	Temperature (°C)	Collecting delay (h)	Testis state (1)	Volume (ml)	Motility (%)
4.1	10	24	L	60	20
3.5	10	36	V	70	65
9	10	48	S	150	85
5.2	10	60	F	70	85
4.7	12.5	24	F	30	40
4.6	12.5	36	L	60	65
2.5	12.5	48	V	25	60
5.2	12.5	60	S	115	70
3.4	15	24	S	55	50
3.9	15	36	F	30	90
5.5	15	48	L	80	40
7.9	15	60	V	135	30
5.1	17.5	24	V	160	5
5.4	17.5	36	S	70	70
3.3	17.5	48	F	30	70
8	17.5	60	L	190	25

L = liquid; V = viscous; S = soft; F = firm.

movement. To avoid bias in the assessment, all measurements were carried out by the same experimenter.

2.4. Statistical analysis

The experimental design was analysed by ANOVA through a General Linear Model procedure (GLM). Where significant differences were shown, a complementary Student–Newman–Keul's test was carried out to determine differences among the experimental groups, and 95% confidence intervals were computed according to Scheffe's method. The fit of the model with the data was checked with a normality test. All these procedures were computed using SAS software (SAS, 1990). The relationship between

Table 2
ANOVA analysis (GLM procedure) of effects of testis state, temperature and delay of semen collection on fresh spermatozoa motility (16 male Siberian sturgeons)

Source of variation	*df*	Sum of squares	*F*-ratio	Probability
Main effects	9	9243.75	15.406	0.0017
Testis state	3	3931.25	19.656	0.0017
Delay of semen collection	3	4306.25	21.531	0.0013
Water temperature	3	1006.25	5.031	0.0446
Residual	6	400		
Total	15	9643.75		

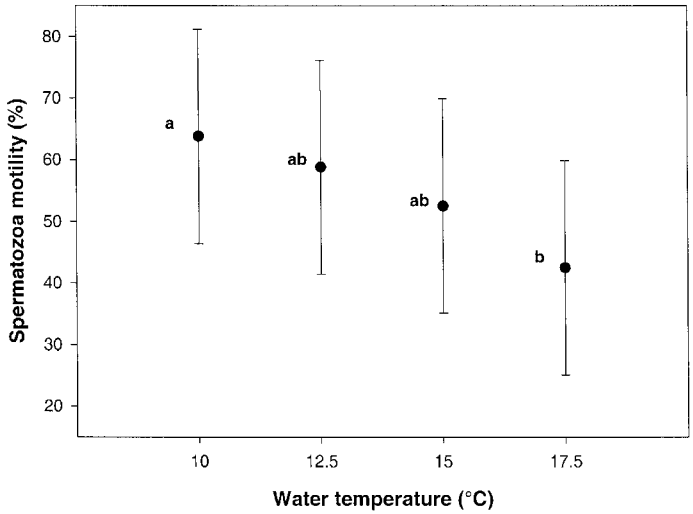


Fig. 1. Dependence of motility of fresh spermatozoa (mean) on water temperature during hormonal treatment, for Siberian sturgeon males reared at 10°C. Results from GLM analysis carried out with 16 males. Vertical bars are 95% confidence interval. Different letters (a and b) correspond to significant difference at $P < 0.05$.

spermatozoa motility and semen characteristics was studied by regression analysis followed by ANOVA of regression and verification of no significant deviation from

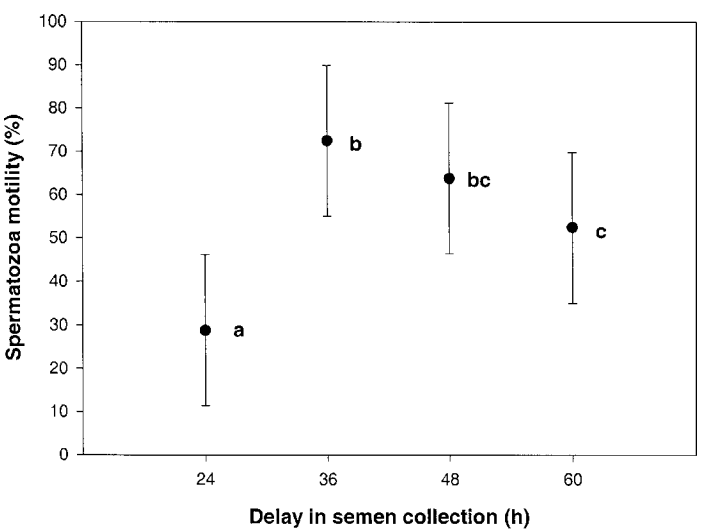


Fig. 2. Dependence of motility of fresh spermatozoa (mean) on the delay in semen collection. Results from GLM analysis carried out with 16 Siberian sturgeon males. Vertical bars are 95% confidence interval. Different letters (a, b and c) correspond to significant difference at $P < 0.05$.

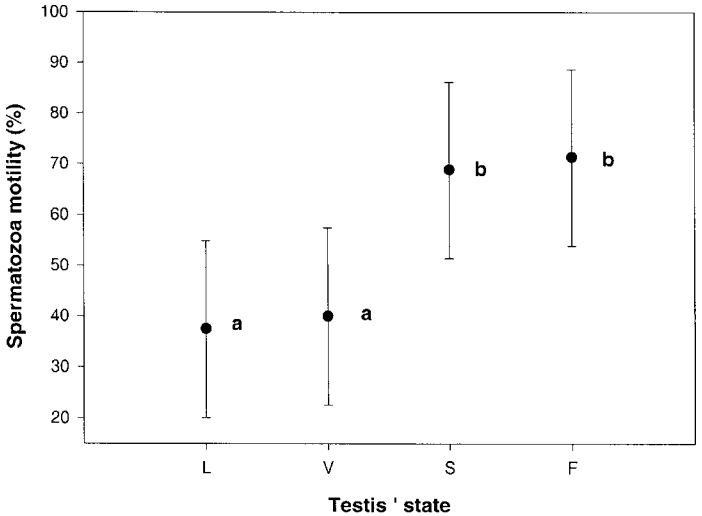


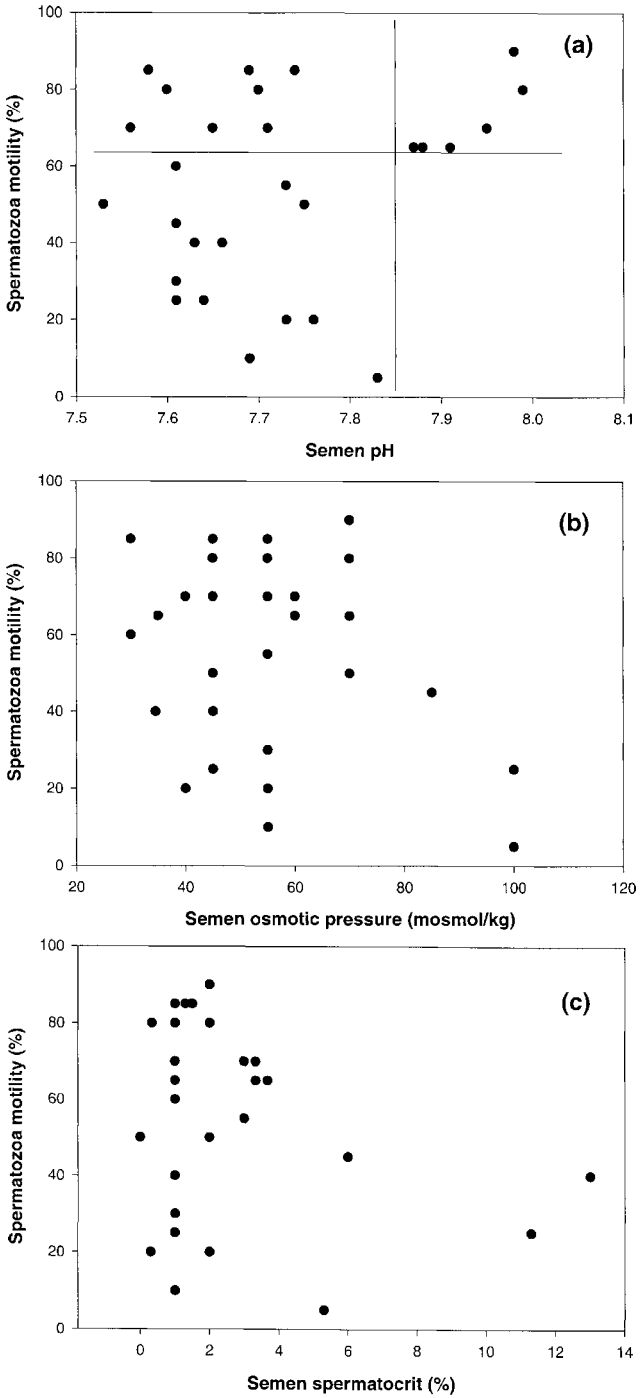
Fig. 3. Dependence of motility of fresh spermatozoa (mean) on the testis state determined 1 month before the experiment. Results from GLM analysis carried out with 16 Siberian sturgeon males. Vertical bars are 95% confidence interval. L = liquid, V = viscous, S = soft, F = firm. Different letters (a and b) correspond to significant difference at $P < 0.05$.

normality with residue analysis (Sigmastat, 1995). Accepted significance levels are: significant, $P < 0.05$; highly significant, $P < 0.001$.

3. Results

Four out of 32 males did not spermiate, one in each group of testis state. As a result of lack of a replicate, assessment of interactions was not possible. GLM analysis was then carried out with spermatozoa motility from only 16 males in order to have a balanced design (Table 1). The only four which spermiated in their respective couple were retained plus 12 others randomly chosen in the remaining pairs. The multiple correlation coefficient (R^2) of the modelling was 0.96 and the results of ANOVA are shown in Table 2. The spermatozoa motility proved to be influenced by the three studied factors, at a significant ($P < 0.045$) level for temperature and at a highly significant level ($P < 0.001$ and $P < 0.002$) for delay of semen collection and testis state, respectively. Figs. 1–3 show representative effects of these three factors. The motility of spermatozoa decreased from 65% to 40% with increasing water temperature during hormonal treatment, with a significant depressive effect at 17.5°C as compared with 10°C (Fig. 1). The motility decreased from 72% to 30% with the delay in semen

Fig. 4. Relationship between fresh spermatozoa motility and semen pH (a), semen osmotic pressure (b) and semen spermatocrit (c) from 28 Siberian sturgeon males.


(a)
Spermatozoa motility (%)
Semen pH
(b)
Spermatozoa motility (%)
Semen osmotic pressure (mosmol/kg)
(c)
Spermatozoa motility (%)
Semen spermatocrit (%)

collection in the following sequence 36, 48, 60 and 24 h (Fig. 2). The better motilities, about 70% as compared with about 40%, were obtained from both males of which the testis were classified as firm and soft in contrast with those having viscous and liquid ones (Fig. 3).

Although there were no statistical relationships between spermatozoa motility and studied semen characteristics from 28 males, scatter plots of the data are shown in Fig. 4. Regardless of the motility, most of the semen pH values ranged from 7.5 to 7.8, but motility above 60% corresponded to pH values higher than 7.85 (Fig. 4a). Motility was not influenced by osmotic pressure, which varied mainly between 30 and 70 mosM/kg; nevertheless, lowest values of motility were related to highest osmotic pressures (100 mosM/kg) (Fig. 4b). Similarly, motility was independent of the density in spermatozoa expressed by spermatocrit; some of the lowest values were even related to highest densities (Fig. 4c).

4. Discussion

This study showed that the spermatozoa motility of cultured Siberian sturgeons is influenced by different factors. Concerning temperature, there are two possible interpretations — either it is the water temperature itself which is important or it is the difference between rearing (10°C in present study) and reproductive temperatures. The finding here of poorest spermatozoa motility at the two highest experimental temperatures (15°C and 17.5°C) was contrary to the optimal range for reproduction described in Section 1. This suggests that it is the difference in temperatures which was tested. The second factor was the delay in semen collection after hormonal injection. A delay of 36 h after stimulation before semen collection clearly provided the most motile spermatozoa as compared with shorter or longer delays. This means that the time schedule for hormonal injection should take into account this result, in contrast with our previous standard practice. The third factor was the maturity state of the male as judged by testis texture hardness 1 month before the experiment. Our findings showed that fish with firmer testes produced the highest motility of spermatozoa. This means that, at the pre-selection time, the males with testis referred to as liquid or viscous had already passed the optimum of sexual maturation, or passed the optimum 1 month prior to hormonal injection. Consequently, the management of males needs to be modified, the males with softer testis can be rejected in November and a final selection should be made just before breeding, as for females. The best motilities, from 70% to 90%, were fairly similar to more recent results on the same species (88% $\pm$ 4%) (Tsvetkova et al., 1996). It is noteworthy that due to the very high value of R^2, the linear modelling approach of the balanced design (16 males) was excellent. This suggests that supposed interactions between the studied factors could not significantly improve the fit of the model.

Among the studied semen characteristics which may influence semen motility, i.e. pH, osmotic pressure and spermatocrit, there was no significant relationship with semen motility. Nevertheless, pH values higher than 7.85 corresponded to motility over 60% and accorded with the finding of Gallis et al. (1991) of better motility at higher pH in that range. Moreover, there were indications of worst motility when spermatocrit was

higher than 10% and osmotic pressure higher than 10 mosM/kg. This latter osmotic pressure effect is in good agreement with results already published on Siberian sturgeon semen (Gallis et al., 1991), in shovelnose sturgeon (*Scaphirynchus platorynchus*) and paddlefish (*Polyodon spathula*) (Linhart et al., 1995).

And finally, in most cases, none of the readily determined semen characteristics tested in the work determined the quality of semen with regard to spermatozoa motility. In this field, further investigations could focus on semen ATP, as it has been recently shown in Siberian sturgeon that energy content fluctuates depending on the origin of semen (Billard et al., 1999).

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